# Correlation of *Borrelia burgdorferi* Sensu Lato Prevalence in Questing *Ixodes ricinus* Ticks with Specific Abiotic Traits in the Western Palearctic[▽][†]

Agustín Estrada-Peña,[1]* Carmelo Ortega,[1] Nely Sánchez,[1] Lorenzo DeSimone,[2] Bertrand Sudre,[2] Jonathan E. Suk,[2] and Jan C. Semenza[2]

*Department of Animal Pathology, Faculty of Veterinary Medicine, University of Zaragoza, Zaragoza, Spain,[1] and European Centre for Disease Control (ECDC), Stockholm, Sweden[2]*

This meta-analysis of reports examining ticks throughout the Western Palearctic region indicates a distinct geographic pattern for *Borrelia burgdorferi* sensu lato prevalence in questing nymphal *Ixodes ricinus* ticks. The greatest prevalence was reported between the 5°E and 25°E longitudes based on an analysis of 123 collection points with 37,940 nymphal tick specimens (87.43% of total nymphs; 56.35% of total ticks in the set of reports over the target area). Climatic traits, such as temperature and vegetation stress, and their seasonality correlated with *Borrelia* prevalence in questing ticks. The greatest prevalence was associated with mild winter, high summer, and low seasonal amplitude of temperatures within the range of the tick vector, higher vegetation indices in the May-June period, and well-connected vegetation patches below a threshold at which rates suddenly drop. Classification of the target territory using a qualitative risk index derived from the abiotic variables produced an indicator of the probability of finding infected ticks in the Western Palearctic region. No specific temporal trends were detected in the reported prevalence. The ranges of the different *B. burgdorferi* sensu lato genospecies showed a pattern of high biodiversity between 4°W and 20°E, partially overlapping the area of highest prevalence in ticks. *Borrelia afzelii* and *Borrelia garinii* are the dominant species in central Europe (east of ~25°E), but *B. garinii* may appear alone at southern latitudes and *Borrelia lusitaniae* is the main indicator species for meridional territories.

Lyme borreliosis (LB) is a tick-borne zoonosis caused by bacteria in the taxonomic complex *Borrelia burgdorferi*. The bacterium is maintained in a horizontal transmission cycle between its vector, ticks of the *Ixodes ricinus* complex, and vertebrate hosts. Larval ticks hatch uninfected (33), and the two subadult stages of the tick life cycle are responsible for the enzootic maintenance of *B. burgdorferi*. The prevalence of LB varies considerably in different European countries, with reported changes in both the spatial and temporal scales of the disease. The distribution of species of *B. burgdorferi* sensu lato (3), prevalence of infected ticks, and the nature of habitats in LB foci (16) show differences within Europe that seem to be relevant to the prevalence of LB, and possibly to the different clinical manifestations of the disease. This issue is particularly important in light of concerns regarding changes in the densities of both vector and reservoirs as a result of climate trends and the potential spread of infection (27, 28).

For *B. burgdorferi* sensu lato to thrive over time in efficient infective cycles, tick larvae must acquire the bacteria from infectious hosts and subsequently transmit them to susceptible host populations as nymphs. The role of adult ticks as vectors is limited in most cases, because adult males do not feed and adult female ticks feed mainly on large animals that are not competent reservoir hosts (15). Climatic forces have been proposed to shape the population structures of pathogens by affecting their tick vectors (26). The host specificity of *B. burgdorferi* sensu lato is a result of negative selection mediated by the alternative complement pathway (24). In Europe, most species or subtypes of *B. burgdorferi* sensu lato are specialized to infect particular groups of vertebrates (24, 25). For example, OspA serotype 2 of *Borrelia afzelii* and OspA serotype 4 of *Borrelia garinii* are associated with rodents and some insectivore species (17, 22), whereas *Borrelia valaisiana* and OspA serotypes 3 and 5 to 8 of *B. garinii* infect terrestrial birds and seabird species (18). Recently, OspA serotype 4 of *B. garinii* has been associated with a new species, *Borrelia bavariensis* (30). Some studies have indicated the influence of tick life cycle traits on the distribution of *B. burgdorferi* sensu stricto genotypes in the United States as a consequence of different climate patterns affecting tick phenology (14). A temperature-regulated pattern of *B. burgdorferi* sensu stricto genotypes in the United States has been interpreted as the control of the synchrony of the activity of the different tick stages by the yearly temperature amplitude and persistence of the pathogen in the primary reservoir host (14). The abundance of ticks is determined not only by climate cues but also by the extrinsic incubation period of spirochetes in ixodid ticks, which equals the duration of development from larva to nymph and is climate sensitive (35). Therefore, patterns of abundance and genotype distribution of *B. burgdorferi* sensu lato populations are shaped substantially by the environmental cues that act on the tick populations, whereas additional layers of complexity are introduced into the system by host population dynamics

* Corresponding author. Mailing address: Department of Animal Pathology (Parasitology), Faculty of Veterinary Medicine, University of Zaragoza, Miguel Servet 177, 50013 Zaragoza, Spain. Phone: 34-976-761-558. Fax: 34-976-761-612. E-mail: aestrada@unizar.es.

and the host immune response to both the bacteria and the ticks (4).

The comparative prevalence and distribution of *B. burgdorferi* sensu lato in Europe have been reviewed (20, 21), and a meta-analysis of the prevalence of *B. burgdorferi* sensu lato genospecies in *I. ricinus* ticks in Europe has already been performed (36). However, these studies focused on a geographic analysis that was based on large administrative divisions, which cannot disentangle the relationships between abiotic traits and patterns of occurrence. Because these abiotic traits are critical drivers of both tick and host populations by regulating seasonal tick activity and density, they should be identified before any potential impact of climate trends on the processes behind the transmission rates of LB can be determined.

Here, we describe a meta-analysis of the reported prevalence and distribution of genospecies of *B. burgdorferi* sensu lato in host-seeking *I. ricinus* ticks in the Western Palearctic region based on a systematic literature review. We describe (i) the distribution of *B. burgdorferi* sensu lato and (ii) occurrence of different *Borrelia* genospecies in *I. ricinus* tick populations in Europe. This analysis is explicitly restricted to the area of distribution of *I. ricinus*, though the bacterium can be transmitted by *Ixodes persulcatus* east of the target area. The following questions concerning the distribution of *B. burgdorferi* sensu lato were examined. (i) How does reporting affect the prevalence, and can this be a confounding factor? (ii) How do the rates of tick infection differ across the surveyed area? (iii) Are these infection rates unambiguously linked to abiotic (temperature, vegetation, landscape) features? (iv) Is there a particular pattern of distribution of *B. burgdorferi* sensu lato species in the Western Palearctic region?

## MATERIALS AND METHODS

**Data collection.** All data on the prevalence of *Borrelia burgdorferi* sensu lato in questing ticks in Europe were obtained from reports published between 1995 and 2009, which are included in the references listed in File S1 in the supplemental material. The methods generally used for detecting *B. burgdorferi* sensu lato in ticks were cultivation in Barbour-Stoenner-Kelly (BSK) medium, dark-field and phase-contrast microscopy, immunofluorescence assay, and PCR. Only reports on prevalence in questing ticks were considered in order to avoid any bias of reported infection in ticks after feeding on putatively infected hosts. Only studies with an explicit mention of the number of specimens examined and prevalence were included in our analysis. If data from different years were reported for the same collection point, the data were averaged for the site for the general meta-analysis and then used as different years for a study of the temporal prevalence trends. As the main purpose of this analysis was to establish whether there is a relationship between the abiotic traits of the habitat and the prevalence of *B. burgdorferi* sensu lato in questing ticks, only papers with reliable coordinates for the collection site or an unambiguous name for the collection locality were included. A total of 236 individual collection localities for *Ixodes ricinus* nymphs and 240 for adults were finally selected. The total number of collection points was 548, including reports reporting prevalence without separation into nymphs and adults. These reports examined a total of 43,393 nymphs and 23,928 adults, the results of which were reported independently, and a total of 7,652 nymphal and adult ticks for which infection rates were not provided separately. Prevalence in males and females was not differentiated. Pools of ticks negative for *B. burgdorferi* sensu lato are rare within the *I. ricinus* range. Only eight studies reported data on negative ticks, representing a total of 23 sites for nymphs (907 specimens; 2% of total nymphs) and 21 sites for adults (820 specimens; 3.3% of total adults), and they were processed with the rest of the data. We sought to define the bias of the reported prevalence in ticks and its significance by applying two tests over the data from the papers listed in File S2 in the supplemental material. The agreement of the different tests or the sensitivity and specificity of the tests was analyzed using Win Episcope 2.0 (38). A kappa value greater than 0.6 was obtained for every comparison of two tests. Thus, we concluded that microscopy is the best technique for detecting positive samples, resulting in a $\pm 2\%$ variation in the reported prevalence. The low bias, together with conclusions from other papers and a previous comparison of the mean prevalence of *B. burgdorferi* sensu lato (36), provided support for using the prevalence as reported.

We classified prevalence into four levels according to the quartiles of its frequency distribution. Reports without explicit indication of the tick stage analyzed were not included. The levels for questing nymphs were <12% (first quartile), 12 to 15% (second quartile), 15 to 20% (third quartile), and >20% (fourth quartile). For adults, the levels were <6%, 6 to 14%, 14 to 23%, and >23% for the first to fourth quartiles, respectively. The reported prevalence of *B. burgdorferi* sensu lato was significantly different between nymphs and adults ($P = 0.0431$), and the standard deviation in nymphs negatively correlated with the number of specimens examined. A higher standard deviation was observed for adults, regardless of the number of specimens. This result has a direct impact on the classification of prevalence in questing ticks into discrete classes, because quartiles are different according to the stage considered. Consequently, further statistical analysis referred only to prevalence in nymphal ticks involving more than 100 specimens, resulting in 123 collection points involving a total of 37,940 nymphs, representing 87.43% of the total nymphs and 56.35% of the total ticks in the entire data set.

**Statistical analysis.** We specifically aimed to determine (i) the link between prevalence and abiotic traits, (ii) the association between reporting patterns of *B. burgdorferi* sensu lato genospecies according to categories of abiotic traits, and (iii) the evolution of prevalence over time.

**Association of prevalence and abiotic traits.** We aimed to associate the prevalence of *B. burgdorferi* sensu lato in questing ticks with variables describing abiotic traits of the habitat, such as temperature, vegetation stress, and landscape connectivity. The collection points alone cannot capture the spatial nature of these putative epidemiological drivers. A circular buffer zone of 0.25° in diameter was created for each reported collection point, which was the geometric center of each buffer zone. The prevalence reported for points inside each buffer was averaged for that buffer zone; thus, the buffer zones were classified according to the prevalence quartiles.

Raw abiotic variables were obtained from a set of monthly satellite images from 2000 to 2009. Basic processing of the temperature images produced the annual mean, absolute minimum and maximum temperatures, mean temperature in the month with absolute minimum, and mean temperature in the month with absolute maximum. For vegetation, we obtained annual mean, minimum, and maximum enhanced vegetation index (EVI) (a standard measure of vegetation greenness to which *I. ricinus* is very sensitive). We computed the final set of monthly variables for the whole period from 2000 to 2009. We also aimed to remove redundant information from satellite imagery and to provide the smallest number of explanatory variables using principal component analysis (PCA). PCA reduction retains the variability of the original explanatory variables and accounts for prevalence rates with a minimal loss of information (8). Full details regarding data acquisition and further processing are available in File S3 in the supplemental material.

Habitat connectivity has been suggested as an important abiotic feature in the epidemiology of some diseases caused by tick-borne pathogens, such as Lyme borreliosis (LB) (2) or Crimean-Congo hemorrhagic fever (12). Because the horizontal movement of ticks occurs mainly while they are feeding on hosts, their movements across the habitat matrix may be the cause of different prevalence rates due either to isolation or the high connectivity of host populations. Thus, we wanted to assess the relationships between habitat connectivity and the reported prevalence of *Borrelia* in ticks. Here we calculated traversability from satellite images, an index directly correlated with habitat connectivity. Full details regarding data acquisition and further processing are available in File S3 in the supplemental material.

We sought to capture an indicator of the probability of finding infected nymphal ticks per area unit based on abiotic features and produce a map of the target area classified according to such indicators. For each prevalence quartile, associated climate traits were used to produce a qualitative evaluation of risk according to Office International des Epizooties (OIE) standards (1, 6) on five levels, namely, high, moderate, low, negligible, and null, which directly correlate with the probability of finding nymphal ticks with prevalence in the four quartiles. The method correctly classified 121 of the 123 buffer zones at 0.25° of spatial resolution. The two erroneously classified buffers were sites belonging to the "negligible" category but classified as "low." We assumed the same predictive power for a map of the complete target area and produced a grid of hexagonal cells at 0.25° of spatial resolution covering the study territory. The climate variables were transferred to each cell, and the risk classification was applied to each cell.
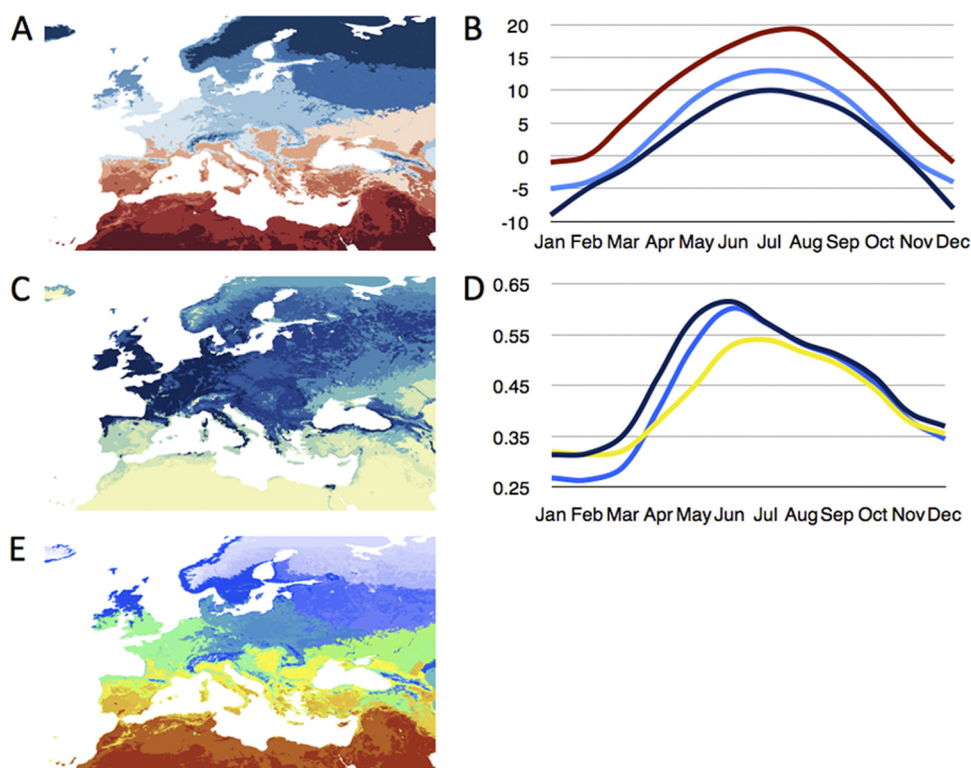
FIG. 1. Computation of Western Palearctic abiotic trait (temperature and vegetation) signatures through a hierarchical cluster analysis. (A and C) Significantly different clusters were obtained for both temperature (A) and vegetation (C) traits using clustering methods based on Mahalanobis distance to the cluster centroid. The clusters are colored randomly. (E) The intersection of both groups of clusters produced a final set of 69 signatures, which are significantly different. Panels B and D show examples of the monthly values in descriptor variables over which signatures are built. (B) Monthly temperature values (°Celsius) were included for three randomly selected signatures, with the colors in the line chart approximating those of panel A. (D) Monthly enhanced vegetation index (EVI) values were included for three randomly chosen signatures, with the colors in the line chart approximating those of panel C. (Copyright, EuroGeographics for the administrative boundaries.)

**Associations between *B. burgdorferi* genospecies and abiotic traits.** The specific aim was to determine the occurrence of the *B. burgdorferi* sensu lato genospecies in the Western Palearctic region according to climate traits. Previous analyses of the distribution of *B. burgdorferi* sensu lato genospecies were based only on point reports and did not account for the ecological reasons underlying such an association. Only genospecies determined by PCR were included for analysis of the reported distribution of *B. burgdorferi* sensu lato species and all records were used for this procedure. The *Borrelia* species considered were *B. burgdorferi* sensu stricto, *B. afzelii*, *B. garinii*, *B. valaisiana*, and *B. lusitaniae*. Only a few records were available for *Borrelia spielmanii* and for the rare *Borrelia bisettii*, so these species were not included in the study. We did not include the newly recognized species *Borrelia bavariensis* (30). Thus, *B. garinii* OspA serotype 4 strains were included as *B. garinii*, because all of the reports included in our data set were published before the new species was named and because most of them did not report OspA serotypes, making it impossible to assign the isolates to *B. garinii* or *B. bavariensis*.

We performed hierarchical agglomerative clustering (HAC), a method that produces a series of clusters with subclusters, which in turn have subclusters, and so forth, based on abiotic (temperature and vegetation) data. All of the clusters produced by this method can be considered discrete hierarchical classifications of continuous temperature and vegetation traits. HAC not only produces spatially coherent information, but it also retains the seasonal information for the original series of images (Fig. 1). We then characterized habitats by the assemblages of *B. burgdorferi* genospecies, with methods based on a correspondence analysis (7). The result is the ordination of the abiotic signatures according to the relative prevalence of each genospecies, weighted by the number of times each genospecies was reported in the target area. All of the procedures adhered to details published previously (11, 19) and are available in File S3 in the supplemental material.

**Prevalence over time.** To compare prevalence over the years, data were merged for ranges of years using 2003 as the cutoff (pre-2003 and post-2003). The

year 2003 was chosen because it marks the midpoint of the study period. Only data on nymphal ticks and from studies with more than 100 analyzed nymphal specimens were used. Analysis of variance (ANOVA) was to compare the merged groups. Other analysis methods produced an atomization of the reports in small groups, which is not adequate for robust comparisons.

## RESULTS

**Geographic range of prevalence rates in questing ticks.** Figure 2 shows the distribution of the prevalence of *B. burgdorferi* sensu lato in questing ticks across latitudes and longitudes using the whole set of 548 collection points with a total of 74,973 examined ticks (both nymphs and adults). Regression curves between prevalence and coordinates (Fig. 2A and B) showed an increase according to latitude and longitude. Notably, a sharp change in prevalence was seen between 5°E and 25°E. Most of the collections were reported within these two longitude points, with only 20 collection points reported east of 25°E. The number of specimens studied for every collection point was variable across the geographic range (Fig. 2D and E). The prevalence was inconstant if a small number of ticks were examined (Fig. 2F). When only reports involving more than 100 nymphs were included, the prevalence in questing ticks was highest in the 5° to 25°E range. This result persisted when original reports were smoothed according to 0.25° buffer zones (Fig. 2G and H).
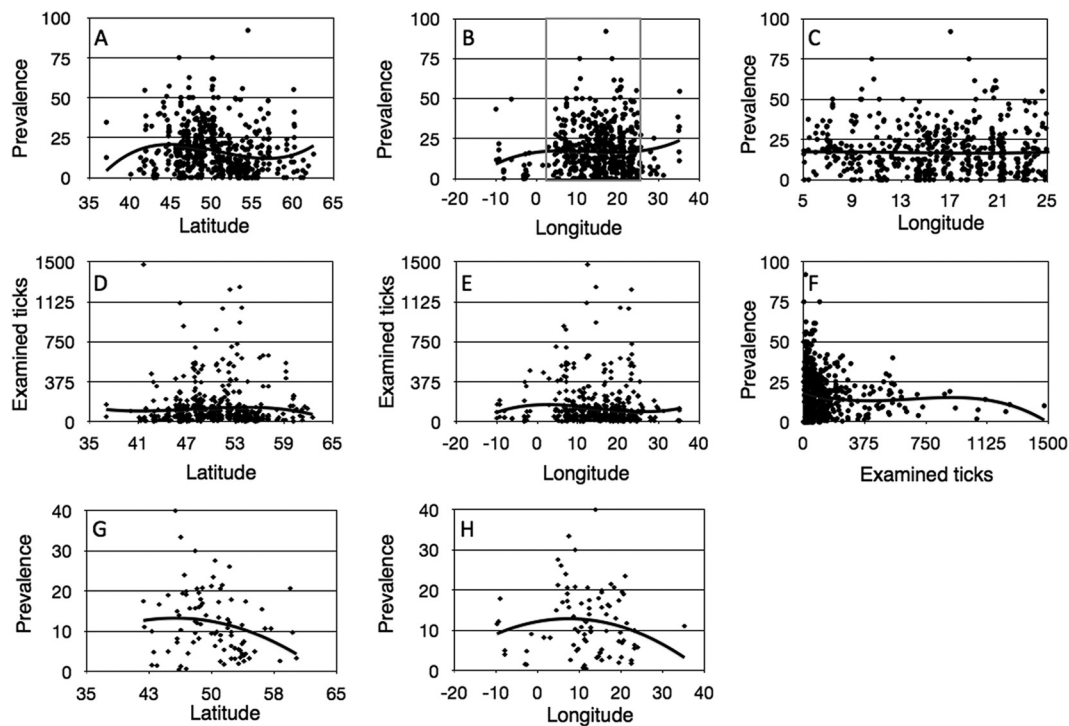
FIG. 2. Reported distribution of *Borrelia burgdorferi* sensu lato prevalence in questing *Ixodes ricinus* ticks according to latitude (A) and longitude (B) for all 548 reported points. The area in gray in panel B is further detailed in panel C to show the area between 5°E and 25°E. In panels D and E, the number of studied ticks is plotted according to latitude and longitude, showing the relationship between the number of studied ticks and the reported prevalence (F). Panels G and H show the distribution of reported prevalence when only reports studying more than 100 nymphal ticks were included in the meta-analysis and the rates were smoothed following 0.25° diameter buffer zones drawn over each report.

**Correlation of prevalence with abiotic traits.** Abiotic variables obtained for 0.25° buffer zones were compared according to the quartiles of prevalence in questing nymphs in Table 1. Analysis of variance (ANOVA) detected significant differences for some variables related to temperature. Absolute annual minimum and mean maximum temperatures had different values for the four prevalence categories. Differences in the absolute maximum temperature were significant only among some categories. Principal component analysis (PCA) reduction of the monthly temperature variables provided a better set of descriptors for categorized prevalence rates; the three axes together were able to separate the four prevalence categories. Values on the first temperature-derived principal axis correlated directly with increased mean temperature according to the four prevalence categories. The second temperature-derived axis was inversely related to the amplitude of the seasonal variability in temperature. Areas in the second and third quartiles correlated with medium values of this variable, whereas areas within the fourth quartile were associated with the lowest values. The third temperature-derived axis is inversely correlated with minimum winter temperatures. Sites in the first quartile tended to be related to areas with the lowest winter temperatures, whereas the rest of the quartiles had correlatively higher values for this axis. These differences were coherent and homogeneous among groups. Variables derived from vegetation features were not adequate descriptors of the ranges of *Borrelia* prevalence if used alone. Traversability values (herein used as a measurement of habitat connectivity)

correlated well with the prevalence quartiles in nymphal ticks. Sites in the first or fourth quartiles of the prevalence distribution had relatively low traversability, whereas the second and third quartiles were linked to sites with higher traversability.

The classification of a 0.25° grid covering the target territory with a qualitative risk analysis based on significant climate variables is shown in Fig. 3A. High risk was observed in areas of central Europe, overlapping the distribution of the reports with highest prevalence in questing ticks. Sites of high risk were consistently linked (Fig. 3B) to sites with relatively low winter temperatures and the highest values in summer within the distribution range of the vector, with a fast temperature increase in summer and slow decrease in autumn. These sites also have low vegetal stress (high enhanced vegetation index [EVI] values), even if this factor was not significant in the statistical analysis. Risk decreased further to "moderate" following a roughly concentric pattern around the previous category. Areas of moderate risk were associated with a pattern of relatively high winter and low summer temperatures, with a clearly lower EVI signal compared to sites with high risk. Areas of low risk were peripheral to the high and moderate ones and mainly characterized by very low temperatures and the smallest EVI signal in winter.

**Distribution of *B. burgdorferi* sensu lato genospecies in the Western Palearctic region.** Figure 4 shows the classification of the climate-derived signatures in the Western Palearctic region according to the association of *B. burgdorferi* sensu lato genospecies. All species appeared to be similarly prevalent over a

TABLE 1. Abiotic variables for the four quartiles of *B. burgdorferi* sensu lato prevalence in *I. ricinus* nymphal ticks grouped according to 0.25° buffer zones[a]

| Variable[b] | Abiotic variables (mean ± SEM)[c] | | | | |
|---|---|---|---|---|---|
| | All samples | 1st quartile | 2nd quartile | 3rd quartile | 4th quartile |
| Temp | | | | | |
| Mean | 281.5 ± 0.2 | 281.9 ± 0.8 A | 280.76 ± 0.2 A | 281.1 ± 0.3 A | 282.3 ± 0.4 A |
| Minimum | 266.8 ± 0.2 | 265.6 ± 0.2 A | 266.1 ± 0.4 B | 268.2 ± 0.3 C | 269.8 ± 1.0 D |
| Mean minimum | 277.3 ± 0.2 | 277.2 ± 0.7 A | 276.8 ± 0.2 A | 277.3 ± 0.3 A | 278.6 ± 0.4 B |
| Mean maximum | 285.8 ± 0.3 | 284.7 ± 0.3 A | 285.5 ± 0.5 A | 286.5 ± 0.9 B | 287.8 ± 0.7 C |
| Maximum | 297.7 ± 0.3 | 297.5 ± 0.9 A | 296.8 ± 0.3 A | 298.0 ± 0.5 A | 299.1 ± 0.9 B |
| EVI | | | | | |
| Mean | 0.43 ± 0.00 | 0.44 ± 0.02 A | 0.43 ± 0.01 A | 0.42 ± 0.01 A | 0.43 ± 0.01 A |
| Maximum | 0.60 ± 0.00 | 0.59 ± 0.01 A | 0.63 ± 0.01 B | 0.59 ± 0.01 A | 0.59 ± 0.01 A |
| Minimum | 0.25 ± 0.01 | 0.32 ± 0.03 A | 0.23 ± 0.01 B | 0.23 ± 0.01 B | 0.26 ± 0.02 C |
| PCA analysis | | | | | |
| Temp | | | | | |
| First PCA axis | 990.4 ± 0.9 | 987.0 ± 1.1 A | 989.5 ± 1.6 A | 993.0 ± 3.0 B | 997.5 ± 2.4 C |
| Second PCA axis | 3.7 ± 0.3 | 9.0 ± 0.7 A | 5.6 ± 0.5 B | 5.2 ± 0.5 B | 2.0 ± 0.6 C |
| Third PCA axis | 2.9 ± 0.2 | 0.2 ± 0.4 A | 3.3 ± 0.3 B | 3.2 ± 0.3 B | 3.6 ± 0.2 B |
| EVI | | | | | |
| First PCA axis | 1.5 ± 0.01 | 1.6 ± 0.05 A | 1.5 ± 0.02 A | 1.5 ± 0.02 A | 1.5 ± 0.04 A |
| Second PCA axis | 0.14 ± 0.01 | 0.03 ± 0.04 A | 0.20 ± 0.02 B | 0.12 ± 0.02 C | 0.14 ± 0.02 C |
| Third PCA axis | 0.09 ± 0.00 | 0.05 ± 0.01 A | 0.11 ± 0.01 B | 0.09 ± 0.01 B | 0.06 ± 0.00 A |
| Traversability | 912.0 ± 40.6 | 730.1 ± 99.3 A | 968.9 ± 48.6 B | 1003.0 ± 80.0 C | 745.6 ± 107.7 A |

[a] Only reports studying more than 100 nymphs were included.
[b] Temperature is given in degrees Kelvin; all other variables are unitless.
[c] Prevalence quartiles are described in detail in Materials and Methods. Values with different letters (A, B, C, and D) had significant differences by ANOVA analysis.

wide area in central and Western Europe between 4°W and 20°E, overlapping with the area of highest prevalence in questing ticks. *B. lusitaniae* was the dominant species reported in meridional portions of the target area, and *B. burgdorferi* sensu stricto was the dominant species in eastern portions of the Western Palearctic. *B. garinii* and *B. lusitaniae* were the most often reported species in parts of Spain, Portugal, Italy, and Turkey. Therefore, the full complement of species is linked to sites with the highest temperatures and smallest vegetal stress. Both *B. lusitaniae* and *B. garinii* are the dominant species in Mediterranean habitats. *B. valaisiana* was an indicator of colder areas and a low vegetation index. *B. valaisiana* alone was associated with large areas where few reports exist, giving a false impression of the dominancy of such species in these areas.

**Temporal prevalence trends in ticks.** No differences were observed in ANOVA analyses carried out on prevalence in nymphs ($n > 100$) for the periods before and after 2003 ($P = 0.651$). Evident, but nonsignificant, differences were found in the number of reports ascribed to the four prevalence rate intervals. More sites had prevalence rates categorized in the second and fourth quartiles post-2003 (19 and 21, respectively) compared to pre-2003 (13 and 16, respectively). An analysis of the abiotic variables associated with the five levels of risk did not show a significant trend.

## DISCUSSION

The prevalence of *Borrelia* infection in questing ticks is one of the most essential components of risk assessment for Lyme

borreliosis (LB) (29). Different approaches from published reports provide variable figures on the geographic distribution of prevalence. These prevalence figures are meaningless without an adequate dissection of the factors delineating them. A meta-analysis that integrates relationships with abiotic variables provides a more versatile choice than the traditional review methods and allows quantitative conclusions to be drawn. Previous studies painted an incomplete picture of prevalence and species distribution in the Western Palearctic region, associating them with administrative divisions in the region, and leading to an incomplete understanding of the reasons behind such a distribution. Our analysis of data from 548 published records in the Western Palearctic region including roughly 74,000 ticks showed that the overall mean prevalence is significantly higher in adult ticks than nymphs, which is in line with previous reports (31). Because we found that a small number of studied ticks explained a higher variability in reported rates, we further filtered the reports to include only those dealing with an adequate number of ticks.

The geographic background of prevalence in ticks showed a significant trend with the highest rates in parts of central and western Europe. This result contradicts previous reports (16, 26, 36) and is probably derived from the way reports were included in previous analyses, which were based only on administrative divisions without further spatial structure or ecological meaning. A significant increase in prevalence from west to east was reported only in adults and attributed to "the high infection rates in central Europe combined with the large number of studies carried out in these regions" (36). In our study, prevalence rates were very different among adults and nymphs,
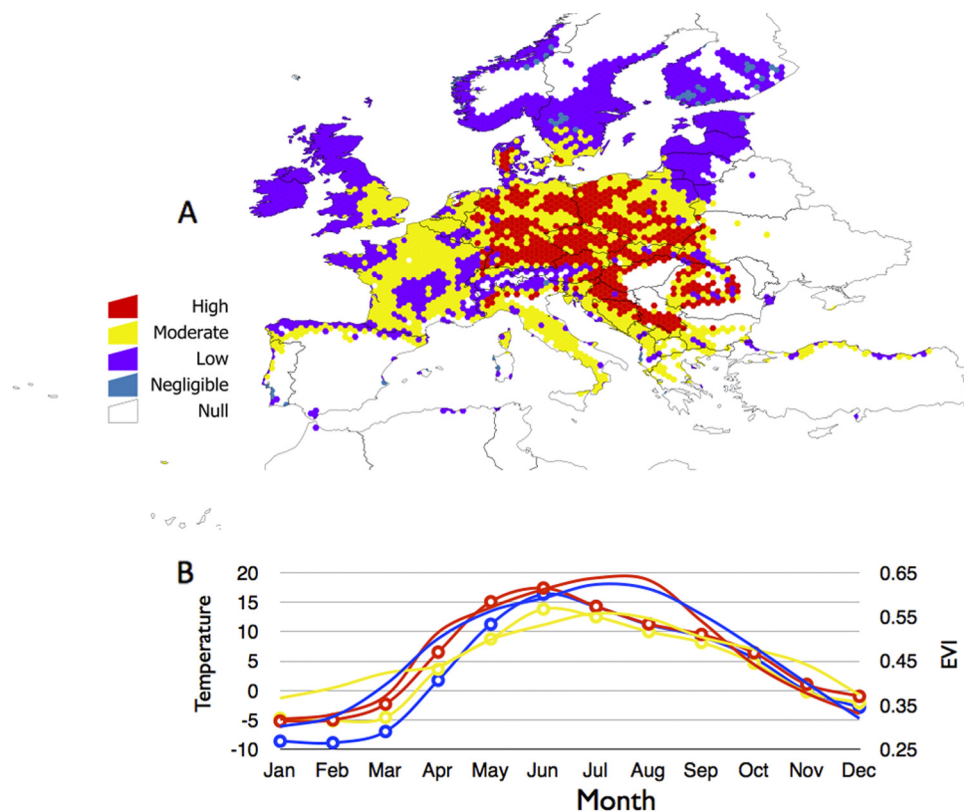
FIG. 3. Classification of the target area according to five qualitative levels of risk built from the quartiles of prevalence rates for *Borrelia burgdorferi* sensu lato in nymphal *Ixodes ricinus* questing ticks. The map is colored according to the risk categories calculated for a grid of 0.25° of spatial resolution. A line chart of the monthly temperature (°Celsius) and EVI values for the three most prominent categories is included. Lines linking circles represent monthly EVI values (right-hand *y* axis), and solid lines represent monthly temperature values (left-hand *y* axis). (Copyright, EuroGeographics for the administrative boundaries.)

which we explain by the fact that host-seeking adult ticks have had two blood meals from different hosts. Thus, the introduction of adults into the analysis, even if examining a significant number of samples, diluted the relationships between the habitat traits and prevalence rates in the tick population because of the confounding effect of additional hosts. The inclusion of only reports examining more than 100 nymphs led to consistent results correlating the relationships between abiotic traits and prevalence while still retaining an adequate number of ticks for analysis. Previous reports of higher prevalence in eastern Europe seem to be the result of studies examining small numbers of ticks, which may be adequate for the local assessment of the presence of *B. burgdorferi* sensu lato but unsatisfactory for the appraisal of prevalence patterns in a wide area.

One of the most relevant results of our study was the relationship between abiotic traits and the prevalence of *B. burgdorferi* sensu lato in nymphal ticks, uncovering a relationship between prevalence and temperature already suggested in previous studies (14, 26). Thus, environmental features of the Western Palearctic region are linked to the geographic pattern of variation in *B. burgdorferi* sensu lato prevalence in ticks. The question is whether these results are biologically meaningful or just statistically significant. Sites with the highest prevalence had intermediate thermal amplitudes and the highest mean and maximum temperatures within the margins suitable for the tick vector. The lowest prevalence rates were associated with

the highest thermal amplitudes and lowest maximum and minimum temperatures. Medium and high prevalence sites were associated with an intermediate winter temperature and low thermal amplitude, resulting in low summer maximum values. These findings are consistently supported by the results of the principal component analysis (PCA) reduction of abiotic variables. Thus, environmental traits indicate that a long season of tick activity (i.e., a long period of low vegetal stress) and a low mortality rate for ticks, as well as a high turnover of host populations, were related to the highest prevalence. Extremely cold temperatures, high thermal variability, and high water saturation deficit (through high vegetal stress or high EVI) are all markers of low tick abundance or short tick seasonal activity, and thus low contact rates between ticks and pathogen reservoirs.

Traversability was used here as a general measurement of habitat connectivity (39). The empirical background showed that habitat connectivity has an impact on tick abundance along a network of connected vegetation patches at the landscape level (10). Small, highly fragmented, not well-connected patches may lead to isolated host and tick populations, perpetuating transmission cycles and sometimes resulting in high prevalence rates because of a lack of connectivity of local hosts with neighboring host populations. Well-connected patches allow a continuous exchange of hosts and, therefore, a circulation of infected ticks and hosts. Both low and very high prevalence rates were observed in ticks collected in zones with low
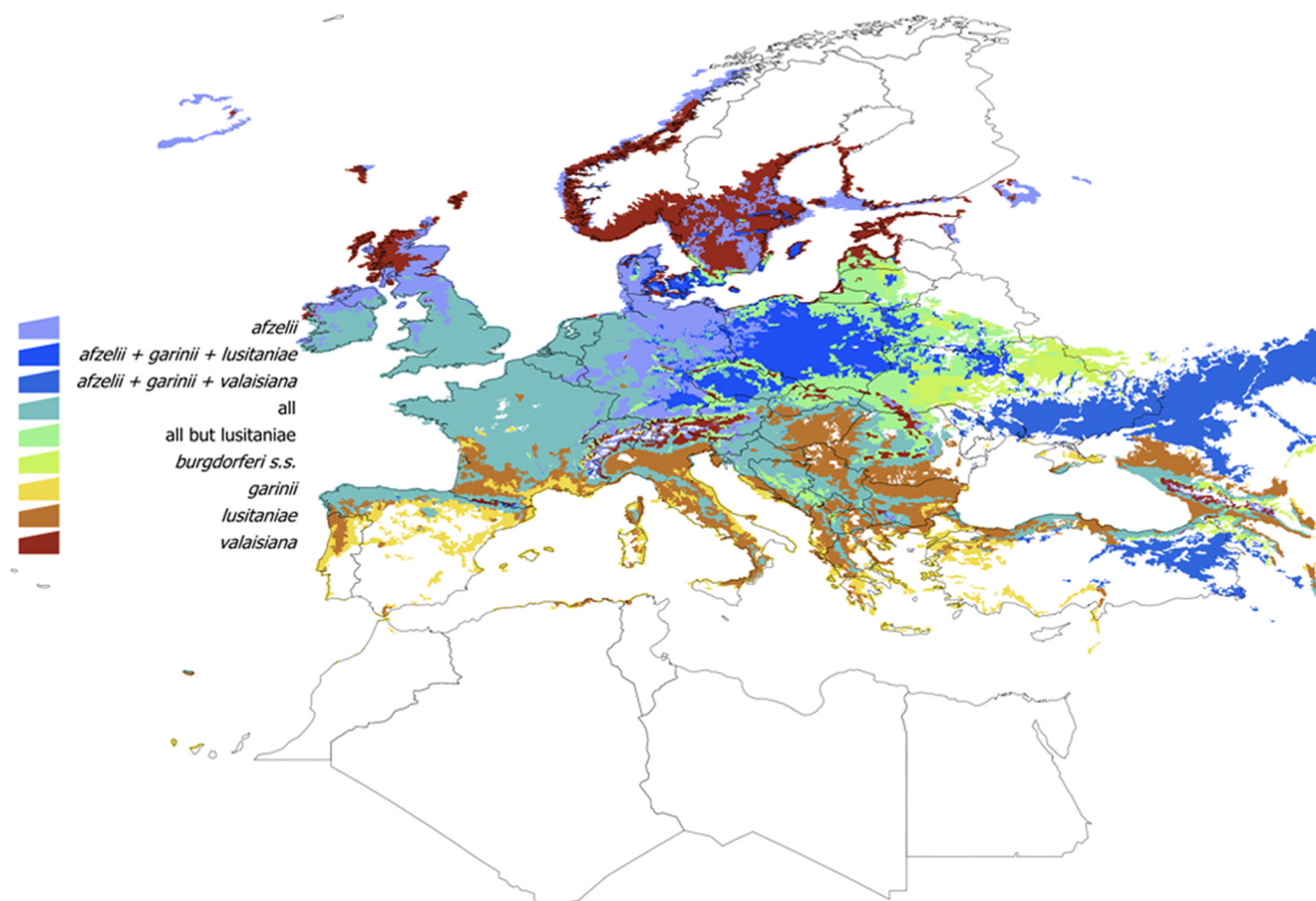
FIG. 4. Distribution of the most representative species of *B. burgdorferi* sensu lato in the Western Palearctic region revealed by correspondence analysis and the indicator species method. The distribution of the *Borrelia* species *B. afzelii*, *B. garinii*, *B. lusitaniae*, *B. valaisiana*, and *B. burgdorferi* sensu stricto (s.s.) alone and in various combinations is shown. The analysis was performed with a total of 548 collection points examining 43,393 nymphs and 23,928 adults, and the set of habitat signatures was developed from monthly temperature and EVI features. (Copyright, EuroGeographics for the administrative boundaries.)

traversability, where both ticks and hosts may be locally common but largely isolated because of a lack of connectivity among patches. Large patches had small connectivity values and, thus, low traversability, covering large portions of the territory and loaded with substantial host populations. Infection rates in ticks are largely "diluted" because the high host densities buffer drastic changes in *B. burgdorferi* sensu lato prevalence. A low connectivity rate for habitat patches is not always synonymous with low prevalence in ticks (32). The classification map of Europe must be regarded as the best explanation of the tick prevalence rates as an output of abiotic variables, and not as a map of risk for humans. While the acarological risk is acknowledged as one of the risk factors for disease (29), it measures only the contact rates of infected ticks and humans, with other factors partially accounting for the incidence rates of the disease in Europe.

Our results pointed to a spatial pattern in the distribution of *B. burgdorferi* sensu lato genospecies in the Western Palearctic region. We used a correspondence analysis modified to find the indicator genospecies that defined signatures of abiotic traits. Interestingly, the area of highest prevalence in nymphal ticks overlapped with the area of highest biodiversity, which is con-

sistent with optimum suitability for the transmission cycle, the area where optimum circumstances for ticks, hosts, and the potential for pathogen survival in both ticks and reservoirs meet. The distribution patterns of *B. burgdorferi* sensu lato genospecies are obviously obscured by the nature and scale of our study but showed a clear link with temperature gradients and vegetation features. These gradients are powerful drivers of tick abundance, seasonality, and the dominance of the different spirochete reservoirs, resulting in highly variable communities of hosts in the targeted region. Our results point to the need for a broad field study addressing the distribution of *B. burgdorferi* sensu lato genospecies (13, 30) as it correlates with the genotypic features of the tick vector and climate signatures developed in this study.

Therefore, *B. burgdorferi* sensu lato can be regarded as a group of different ecological populations, or ecotypes, whose distribution and abundance are shaped by climatic forces, which also drive tick vector dynamics, together with the complex layers resulting from the host species and abundance patterns. Abiotic factors, such as temperature, atmospheric saturation deficit, and hours of light, are well-known to affect the host-seeking behavior and phenology of ticks, providing

the variability in tick life cycle according to geographic range (34). Theoretical studies indicate that these complexities probably yield wide spatial-temporal fluctuations in the relative abundance of different spirochete genotypes (5). The prevalence and distribution of genospecies as related to abiotic signatures show a correlation with the geographic range of definite features of the cuticular hydrocarbon composition of *I. ricinus* (9) and with the range of expansion after the last glaciation in the Western Palearctic region (37). However, recent studies have stressed that *I. ricinus* lacks a genetic structure for its populations (23). Though the structure of host communities determines the population structure of *B. burgdorferi* sensu lato genotypes in Europe (13), with abiotic traits as a "proxy" of such a distribution, we hypothesize that climate signatures have a definite impact on the distribution of different "strains" of *I. ricinus*, thus modulating *B. burgdorferi* sensu lato prevalence.

Our meta-analysis was not intended to capture the evolutionary and ecological details responsible for the exact distribution of *B. burgdorferi* sensu lato; we sought to assess the rough distribution of prevalence rates in questing ticks along the main climatic gradients in the Western Palearctic region as a first step to addressing changes in the risk of infection for humans. The background noise introduced by the precise timing and methods of collection, which lack representativeness of the complete landscape around the collection point and the whole period of tick activity in most cases, probably obscures more clear patterns of association between prevalence and abiotic traits. The dynamic nature of the drivers detected with this meta-analysis is undoubtedly behind the rather noisy cycles of variation in both space and time. How the long- and short-term variability of climatic forces delineate regions with different epidemiological contexts is still unknown.

### REFERENCES

1. **Agence Française de Sécurité Sanitaire des Aliments (AFSSA).** 2009. Une métode qualitative d'estimation du risque en santé animale. Agence Française de Sécurité Sanitaire des Aliments (AFSSA), Bialec-Nancy, France.
2. **Brownstein, J. S., D. K. Skelly, T. R. Holford, and D. Fish.** 2005. Forest fragmentation predicts local scale heterogeneity of Lyme disease risk. Oecologia **146:**469–475.
3. **Derdáková, M., and D. Lencáková.** 2005. Association of genetic variability within the *Borrelia burgdorferi* sensu lato with the ecology and epidemiology of Lyme borreliosis in Europe. Ann. Agric. Environ. Med. **12:**165–172.
4. **Dizij, A., and K. Kurtenbach.** 1995. *Clethrionomys glareolus*, but not *Apodemus flavicollis*, acquires resistance to *Ixodes ricinus* (Acari: Ixodidae), the main European vector of *Borrelia burgdorferi*. Parasite Immunol. **17:**177–183.
5. **Dobson, A.** 2004. Population dynamics of pathogens with multiple host species. Am. Nat. **164:**64–78.
6. **Dufour, B., and R. Pouillot.** 2002. Aproche qualitative du risque. Epidemiol. Santé Anim. **41:**35–43.
7. **Dufrêne, M., and P. Legendre.** 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol. Monogr. **67:**345–366.
8. **Eastman, J. R., and M. Fulk.** 1993. Long sequence time series evaluation using standardized principal components. Photogram. Eng. Remote Sens. **59:**991–996.
9. **Estrada-Peña, A., et al.** 1998. *Ixodes ricinus* strains in Europe. Zentralbl. Bakteriol. **287:**185–189.
10. **Estrada-Peña, A.** 2003. The relationships between habitat topology, critical scales of connectivity and tick abundance *Ixodes ricinus* in a heterogeneous landscape in northern Spain. Ecography **26:**661–671.
11. **Estrada-Peña, A., J. M. Venzal, and C. Sánchez Acedo.** 2006. The tick *Ixodes ricinus*: distribution and climate preferences in the western Palearctic. Med. Vet. Entomol. **20:**189–197.
12. **Estrada-Peña, A., Z. Vatansever, A. Gargili, and O. Ergonul.** 2010. The trend towards habitat fragmentation is the key factor driving the spread of Crimean-Congo haemorrhagic fever. Epidemiol. Infect. **138:**1194–1203.
13. **Etti, S., et al.** 2003. Habitat-specific diversity of *Borrelia burgdorferi* sensu lato in Europe, exemplified by data from Latvia. Appl. Environ. Microbiol. **69:**3008–3010.
14. **Gatewood, A. G., et al.** 2009. Climate and tick seasonality are predictors of *Borrelia burgdorferi* genotype distribution. Appl. Environ. Microbiol. **75:**2476–2483.
15. **Gern, L., and P. F. Humair.** 2002. Ecology of *Borrelia burgdorferi* sensu lato in Europe, p. 149–174. *In* J. S. Gray, O. Kahl, R. S. Lane, and G. Stanek (ed.), Lyme borreliosis: biology of the infectious agents and epidemiology of disease. CABI Publishing, Wallingford, Oxfordshire, United Kingdom.
16. **Gray, J. S., et al.** 1998. Lyme borreliosis habitat assessment. Zentralbl. Bakteriol. **287:**211–228.
17. **Hanincová, K., et al.** 2003. Association of *Borrelia afzelii* with rodents in Europe. Parasitology **126:**11–20.
18. **Hanincová, K., et al.** 2003. Association of *Borrelia garinii* and *B. valaisiana* with songbirds in Slovakia. Appl. Environ. Microbiol. **69:**2825–2830.
19. **Hargrove, W. W., and F. M. Hoffman.** 2004. Potential of multivariate quantitative methods for delineation and visualization of ecoregions. Environ. Manage. **34**(Suppl. 1)**:**S39–S60.
20. **Hubalek, Z., and J. Halouzka.** 1997. Distribution of *Borrelia burgdorferi* sensu lato genomic groups in Europe, a review. Eur. J. Epidemiol. **13:**951–957.
21. **Hubalek, Z., and J. Halouzka.** 1998. Prevalence rates of *Borrelia burgdorferi* sensu lato in host-seeking *Ixodes ricinus* ticks in Europe. Parasitol. Res. **84:**167–172.
22. **Huegli, D., C. M. Hu, P. F. Humair, B. Wilske, and J. Gern.** 2002. *Apodemus* species mice are reservoir hosts of *Borrelia garinii* OspA serotype 4 in Switzerland. J. Clin. Microbiol. **40:**4735–4737.
23. **Kempf, F., K. D. McCoy, and T. De Meeus.** 2010. Wahlund effects and sex-biased dispersal in *Ixodes ricinus*, the European vector of Lyme borreliosis: new tools for old data. Infect. Genet. Evol. **10:**989–997.
24. **Kurtenbach, K., H.-S. Sewell, N. Ogden, S. E. Randolph, and P. A. Nuttall.** 1998. Serum complement sensitivity as a key factor in Lyme disease ecology. Infect. Immun. **66:**1248–1251.
25. **Kurtenbach, K., S. M. Schäfer, S. De Michelis, S. Etti, and H. S. Sewell.** 2002. *Borrelia burgdorferi* sensu latu in the vertebrate host, p. 117–148. *In* J. S. Gray, O. Kahl, R. S. Lane, and G. Stanek (ed.), Lyme borreliosis: biology of the infectious agents and epidemiology of disease. CABI Publishing, Wallingford, Oxfordshire, United Kingdom.
26. **Kurtenbach, K., et al.** 2006. Fundamental processes in the evolutionary ecology of Lyme borreliosis. Nat. Rev. Microbiol. **10:**1038–1475.
27. **Lindgren, E., and T. G. T. Jaenson.** 2006. Lyme borreliosis in Europe: influences of climate and climate change, epidemiology, ecology and adaptation measures. World Health Organization (WHO) Regional Office for Europe, Copenhagen, Denmark.
28. **Lindgren, E., K. L. Ebi, and M. Johanneson.** 2010. Climate change and communicable diseases in the EU Member States. Handbook for national vulnerability, impact and adaptation assessments. ECDC technical document. European Centre for Disease Prevention and Control, Stockholm, Sweden.
29. **Mannelli, A., et al.** 2003. Acarological risk of exposure to agents of tick-borne zoonoses in the first recognized Italian focus of Lyme borreliosis. Epidemiol. Infect. **131:**1139–1147.
30. **Margos, G., et al.** 2008. MLST of housekeeping genes captures geographic population structure and suggests a European origin of *Borrelia burgdorferi*. Proc. Natl. Acad. Sci. U. S. A. **105:**8730–8735.
31. **Ostfeld, R. S., C. D. Canham, K. Oggenfuss, R. J. Winchcombe, and F. Keesing.** 2006. Climate, deer, rodents, and acorns as determinants of variation in Lyme-disease risk. PLoS Biol. **4**(6)**:**e145.
32. **Pardini, R., A. A. Bueno, T. A. Gardner, P. I. Prado, and J. P. Metzger.** 2010. Beyond the fragmentation threshold hypothesis: regime shifts in biodiversity across fragmented landscapes. PLoS One **5**(10)**:**e13666.
33. **Patrican, L.** 1997. Absence of Lyme disease spirochetes in larval progeny of naturally infected *Ixodes scapularis* (Acari: Ixodidae) fed on dogs, J. Med. Entomol. **34:**52–55.
34. **Randolph, S. E.** 1998. Ticks are not insects: consequences of contrasting vector biology for transmission potential. Parasitol. Today **14:**186–192.
35. **Randolph, S. E.** 2000. Ticks and tick-borne disease systems in space and from space. Adv. Parasitol. **47:**217–243.
36. **Rauter, C., and T. Hartung.** 2005. Prevalence of *Borrelia burgdorferi* sensu lato genospecies in *Ixodes ricinus* ticks in Europe: a metaanalysis. Appl. Environ. Microbiol. **71:**7203–7216.
37. **Schmitt, T.** 2007. Molecular biogeography of Europe: Pleistocene cycles and postglacial trends. Front. Zool. **4:**11.
38. **Thrusfield, M., C. Ortega, I. De Blas, J. Noordjuizen, and K. Frankena.** 2001. Winepiscope 2.0: improved epidemiological software for veterinary medicine. Vet. Rec. **148:**567–572.
39. **Urban, D., and T. Keitt.** 2001. Landscape connectivity: a graph-theoretic perspective. Ecology **82:**1205–1218.